Phytochemical, antioxidant and hepatoprotective effects of different fractions of *Moringa oleifera* leaves methanol extract against liver injury in animal model

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ABSTRACT

Objective: To evaluate the potential antioxidant and hepatoprotective effects of *n*-hexane, dichloromethane(DCM), ethyl acetate(EtOAc), *n*-butanol and aqueous fractions of *Moringa oleifera(M. oleifera)* leaves methanol extract against carbon tetrachloride(CCl$_4$)-induced liver injury in rats. **Methods:** These fractions were prepared from the *M. oleifera* leaves methanol extract by solubilization in water and partitioning in *n*-hexane, EtOAc, DCM and *n*-butanol. Their phyto-components were identified by GC-MS analysis. The *in vitro* antioxidant effect of these fractions was carried out by assessment of 1,1-diphenyl-2-picrylhydrazyl scavenging activity. A total of 40 Sprague Dawley rats were allocated into 8 equal groups: group 1 given olive oil (1 mL/kg b.wt.), group 2 injected with CCl$_4$, group 3 to 7 administered with *n*-hexane, DCM, EtOAc, *n*-butanol and aqueous fractions, respectively after CCl$_4$, group 8 administered with silymarin after CCl$_4$. The activities of aspartate aminotransferase, alanine aminotransferase, and the levels of total cholesterol, triglycerides, glucose, total proteins and albumin in serum were determined spectrophotometrically. Glutathione reduced, lipid peroxide by-products levels, glutathione-s-transferase and catalase enzyme activities in the liver homogenate were determined by spectrophotometer. Liver specimens were also examined for histopathological alterations under light microscope. **Results:** The GC-MS analysis of different fractions of the *M. oleifera* leaves methanol extract revealed that *n*-hexane, DCM, EtOAc, *n*-butanol, and aqueous fractions contained 17, 22, 23, 19 and 32 compounds, respectively. The percent and the molecular structure of each component in each fraction were identified. The *n*-butanol and EtOAc fractions exhibited the strongest *in vitro* antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl. CCl$_4$ significantly decreased glutathione reduced and total proteins concentration and glutathione-s-transferase and catalase activities but increased lipid peroxide by-products and total cholesterol levels. The *n*-hexane followed by aqueous and DCM fractions were the most potent to regulate serum enzyme activities and lipid peroxide by-products levels in the liver homogenate. **Conclusions:** *n*-hexane, DCM, and aqueous fractions have the highest effectiveness against CCl$_4$-induced hepatotoxicity. Isolation and purification of the active constituents require further experiments.

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1. Introduction

Hepatic dysfunctions particularly those caused by toxic chemicals, certain drugs, and environmental pollutants continue to be among the principal threats to public health worldwide[1]. In spite of a large number of therapeutic agents, there are no completely safe and effective drugs that maintain hepatic function, or aid in regenerating hepatocytes[1,2]. Thus, alternative pharmaceuticals for the treatment of hepatic dysfunction are highly required. Medicinal plants are promising sources for drugs used for treatment of many diseases. The widely cultivated *Moringa oleifera* (*M. oleifera*, Moringa or drumstick tree) is one of these plants. It was used by the ancient Romans, Greeks, Egyptians, south east and tropical Asian countries, Africa, and Latin America[3]. It contains abundant amount of proteins, fats, beta-carotene, vitamin C, iron, potassium, and other nutrients[3]. It was used since long time ago for treatment of many diseases, and therefore, it is called a "miracle vegetable"[4,5]. For these reasons, this plant has been studied for its various biological activities, including anti-atherosclerotic[6], anti-cardiovascular diseases[7], antiviral[8], antioxidant[9,10], antimicrobial anti-inflammatory[9] and anticancer effects[11,12]. Only few studies have reported the hepatoprotective effects of *M. oleifera* leaves extract. The hepatoprotective action of *M. oleifera* seeds against carbon tetrachloride(CCl_4)-induced liver dysfunction has been reported[13]. The hepatoprotective effect of ethanol extract of *M. oleifera* leaves on liver damage induced by anti-tubercular drugs was evaluated in rats[14]. The CCl_4 model resembles all important properties of animal liver injury, including inflammation, regeneration and fiber formation[15]. Since the leaves are usually the most edible part of this plant, the effect of *M. oleifera* leaves extract against CCl_4-induced liver fibrosis was tested. In a previous study[16] a promising hepatoprotective effect of the crude methanol extract of *M. oleifera* leaves against CCl_4 intoxication was reported. It was the aim of this study and in continuation of those findings, different fractions of hydro-methanol extract of *M. oleifera* leaves were prepared using solvents with increasing polarity namely: *n*-hexane, dichloromethane (DCM), *n*-butanol, ethyl acetate (EtOAc) and finally the aqueous fraction. The antioxidant and hepatoprotective effect of these fractions against CCl_4-induced liver injury in rats were tested.

2. Materials and methods

This experiment was carried out according to the guidelines of the Institutional Animal Care and Use Committee, Cairo University, Approval Protocol No.: CU-II-F-59-15 on 12/2015.

2.1. Plant material and fractionation

The air dried *M. oleifera* leaves (Family: Moringaceae) were obtained from the Egyptian Scientific Society for Moringa at National Research Centre, Dokki, Giza. Leaves were grounded to powder and kept in closed bottle. A voucher sample of *M. oleifera* leaves was kept in the Pharmacology Department, Faculty of Veterinary Medicine, Cairo University. Dried powder was macerated several times at room temperature for one week with methanol (1:10) and filtered. The solvent was removed under vacuum at 40 ℃ by rotary evaporator. *M. oleifera* leaves methanol extract (MOLME) of the plant was stored at −4 ℃. MOLME was fractionated by solubilisation in water and then sequential partitioning using solvents with increasing polarity, namely: *n*-hexane, EtOAc, DCM and *n*-butanol according to the method of Mojarrab *et al*[17]. The fractions and the remaining aqueous fraction were evaporated to dryness and used for phytochemical and pharmacological studies. The yield ratios of each fraction were: 35.0%, 5.5%, 0.7%, 12.0% and 46.8% for *n*-hexane, DCM, EtOAc, *n*-butanol and aqueous fraction, respectively.

2.2. GC/MS analysis

GC (Agilent Technologies 7890A) interfaced with a mass-selective detector (Agilent 7000 Triple Quad) was used. Agilent HP-5ms capillary column (30 m × 0.25 mm ID and 0.25 μm film thickness) was used. The flow rate was 1 mL/min. The injector and detector temperatures were 200 ℃ and 250 ℃, respectively. The acquisition mass range was 50-600. The formulae of the components as identified by comparing their mass spectra and RT with those of NIST and WILEY library were recorded.

2.3. In vitro antioxidant effect of different fractions of MOLME

The *in vitro* antioxidant effect of different fractions of MOLME was carried out by assessment of free radical scavenging activity of DPPH as described by White *et al*[18].

2.4. In vivo antioxidant and hepatoprotective effects

2.4.1. Animals

Forty Sprague Dawley 6 weeks' old rats (120–150 g) of both sexes were purchased from Animal Breeding House, NRC, Giza, Egypt. They were kept under hygienic conditions in cages at (25±3) ℃ room temperature with 12 h dark/light cycle. They were allowed free access to standard feed and water. After two weeks of acclimatization period, rats were allocated into 8 equal groups. Rats of the group 1 were given olive oil (1 mL/kg b.wt.) 4 d per week. Rats of group 2 were injected with CCl_4 (30% in olive oil) at a dose of 3 mL/kg.b.wt. intraperitonealy (I/P) twice a week. Rats of group 3 to 7 were administered with *n*-hexane, DCM, EtOAc, *n*-butanol and aqueous fraction, respectively at a dose of 100 mg/kg b.wt. orally in olive oil 24 h after CCl_4. Rats in group 8 were administered silymarin (a reference drug) orally in a dose of 100 mg/kg b.wt. in olive oil 24 h after CCl_4.Treatment persisted for 4 weeks.

2.4.2. Blood samples

Blood samples were collected at the end of the experiment by puncture of retro-orbital plexus of veins of each rat 24 h after the last hepatotoxin administration early before diet. Blood samples were

collected in plain tubes, without anti-coagulant to separate clear serum. Sera were kept at -20 ℃ until used for biochemical analysis.

2.4.3. Serum biochemical assessments

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and the levels of total cholesterol, triglycerides, glucose, total proteins and albumin were determined spectrophotometrically (T80 UV/VIS PG instrument Ltd, UK) using commercial test kits from bioMérieux-France.

2.4.4. Assessment of oxidant/antioxidant markers in liver tissue

All rats were euthanized at the end of the experiment (4 weeks), using over dose of chloroform. Liver specimen of each rat was kept immediately in 10% formalin for histopathological examination and the remaining part was kept at -80 ℃ for preparation of liver tissue homogenate. After washing in ice-cold 0.9% NaCl, one gram of liver tissue of each rat was homogenized in ice-cold potassium chloride solution (1.15% in 50 mM potassium phosphate buffer solution, pH 7.4) using Sonicator (4710 Ultrasonics Homogenizer, USA) to obtain 10% (W/V) liver homogenate which was centrifuged at 4 000 rpm for 5 min at 4 ℃. The supernatant was collected and used for determination of the antioxidant markers.

Glutathione reduced (GSH), lipid peroxide by-products (LPx) levels, glutathione-s-transferase (GST) and catalase (CAT) enzyme activities were determined in the liver homogenate. Spectrophotometer (T80 UV/VIS PG instrument Ltd, UK) was used for analysis of all parameters using commercial test kits from Biodiagnostic Co, Egypt.

Liver specimens were fixed in 10% formol saline then dehydrated, cleared, and embedded in paraffin blocks. Paraffin sections of 5 microns thickness were prepared, stained by hematoxylin and eosin (H&E), and examined for histopathological alterations under light microscope[19].

2.5. Statistical analysis

Data were presented as mean±SE. Differences between means were tested for significance by ANOVA test using SPSS version 16 computer program. Probability level of $P<0.05$ was taken as indication of significance.

3. Results

3.1. Phytochemical analysis

GC-MS of different fractions of the MOLME are illustrated in Figure 1. GC-MS analysis of different fractions of the MOLME revealed that n-hexane, DCM, EtOAc, n-butanol, and aqueous fractions of MOLME contained 17, 22, 23, 19 and 32 compounds, respectively. The major components (>5%) in the n-hexane fraction were (11Z)-10-methyl-11-tetradecenyl propionate and egtazic acid; in the DCM fraction, the compounds were camphoric acid,

(11Z)-10-methyl-11-tetradecenyl propionate, β-orcinaldehyde and egtazic acid; in the EtOAc fraction, the compounds were methyl 3, 6-anhydro-α-D-galactoside, 1-methylcyclohexanol, valine, cis, cis-muconic acid and methylmalonic acid; in the n-butanol fraction, the compounds were betaine, camphoric acid, 3-deoxy-d-mannoic lactone and mannosamine and in the aqueous fractions, the compounds were 6-octadecenoic acid, methyl elucidate, (11Z)-10-methyl-11-tetradecenyl propionate, flavoxate and citrulline.
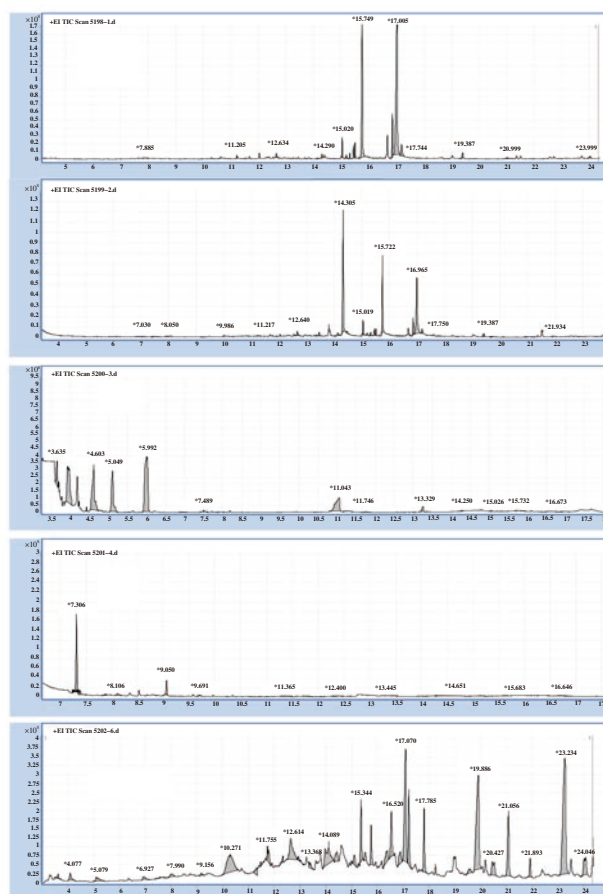
Figure 1. GC-MS peaks of n-hexane fraction (A), dichloromethane (B), ethyl acetate (C), n-butanol (D) and aqueous (D) fractions of the methanol extract of *M. oleifera leaves*.

3.2. Antioxidant effect

3.2.1. In vitro DPPH free radical scavenging activity

The n-butanol and EtOAc fractions exhibited the strongest free radical scavenging activity against DPPH (89.9% and 88.9%, respectively). Mild effects were recorded for n-hexane, aqueous and DCM fractions (24.5%, 26.1% and 13.3%, respectively).

3.2.2. Effect of different fractions of MOLME on serum biochemical parameters

CCl$_4$ significantly ($P<0.05$) increased ALT and AST activities and total cholesterol levels and slightly increased glucose level. However, triglycerides level was slightly decreased. Administration

of the *n*-hexane fraction modulated all the CCl_4-induced biochemical changes. Moreover, DCM fraction and silymarin modulated the CCl_4-induced changes in transaminases activity and triglycerides levels. EtOAc fraction normalized triglycerides and total cholesterol, while *n*-butanol fraction significantly increased triglycerides and decreased blood glucose levels as compared to CCl_4-treated rats. The administration of the aqueous fraction normalized all the biochemical parameters except AST activity (Table 1).

Table 1

Effect of different fractions of *M. oleifera* leaves methanol extract and silymarin on enzymes activity, total cholesterol and triglycerides in serum of rats treated with CCl_4-induced hepatotoxicity (Means±SE, *n*=5).

Groups	AST (IU/L)	ALT (IU/L)	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	Glucose (mg/dL)
Control	101.70±3.52[d]	36.33±1.71[f]	50.76±4.04[de]	54.97±3.44[cd]	79.50±1.10[abc]
CCl_4 alone	151.32±9.24[bc]	166.06±12.24[b]	70.01±5.71[abc]	48.97±2.41[d]	88.57±3.60[ab]
n-hexane	142.74±4.11[bc]	128.25±7.29[c]	64.69±3.63[bc]	66.58±4.62[abc]	54.24±3.46[d]
DCM	124.82±7.60[cd]	100.70±6.98[d]	72.13±2.77[ab]	81.81±2.57[a]	86.71±5.07[ab]
EtOAc	207.35±14.81[a]	227.87±8.29[a]	56.95±3.86[cd]	69.66±9.34[abc]	79.96±3.86[abc]
n-butanol	210.45±16.49[a]	150.94±14.48[bc]	70.07±5.82[abc]	76.48±7.48[ab]	67.23±9.91[cd]
Aqueous	164.16±11.41[b]	137.60±7.38[c]	42.77±3.87[e]	54.85±2.78[cd]	71.74±8.04[bcd]
Silymarin	141.08±6.04[bc]	61.26±1.99[e]	79.54±3.02[a]	63.50±3.00[bcd]	97.87±8.05[a]

Means with different superscripts in the same column are significantly different at $P<0.05$. AST = Aspartate aminotransferase. ALT = Alanine aminotransferase. DCM: Dichloromethane. EtOAc: ethyl acetate.

CCl_4 significantly decreased total globulins and slightly decreased total proteins. However, it significantly increased A/G ratio but it did not alter albumin level. Administration of different fractions of MOLME revealed that *n*-hexane, DCM, EtOAc, *n*-butanol and aqueous fraction as well as the reference drug silymarin normalized the total proteins levels but had no significant change in the profile of other protein parameters (Table 2).

Table 2

Effect of different fractions of *M. oleifera* leaves methanol extract and silymarin on serum proteins profile of rats treated with CCl_4-induced hepatotoxicity (Means±SE, *n*=5).

Groups	Total proteins (g/dL)	Albumin (g/dL)	Total globulins (g/dL)	A/G ratio
Control	7.34±0.23[abc]	3.67±0.26[ab]	3.66±0.13[b]	1.01±0.10[bc]
CCl_4 alone	6.75±0.22[cd]	3.71±0.06[ab]	3.03±0.26[c]	1.27±0.11[a]
n-hexane	6.71±0.16[d]	3.03±0.44[c]	3.76±0.10[b]	0.74±0.09[d]
DCM	7.85±0.09[a]	3.51±0.11[ab]	4.34±0.12[a]	0.82±0.07[cd]
EtOAc	7.09±0.19[bcd]	3.32±0.11[bc]	3.77±0.17[b]	0.89±0.06[cd]
n-butanol	7.42±0.09[ab]	3.60±0.11[ab]	3.82±0.07[b]	0.95±0.04[bcd]
Aqueous	7.06±0.30[bcd]	3.74±0.14[ab]	3.32±0.25[bc]	1.15±0.10[ab]
Silymarin	7.54±0.16[ab]	3.85±0.06[a]	3.69±0.12[b]	1.05±0.03[abc]

Means with Different superscripts in the same column are significantly different at $P<0.05$. DCM: Dichloromethane. EtOAc: ethylacetate.

3.2.3. Antioxidant effect of different fractions of MOLME

CCl_4 significantly decreased GSH concentration and hepatic GST and CAT activities as compared to those of control group but induced significant ($P<0.01$) increase in LPx level. The administration of *n*-hexane, DCM or aqueous fraction of MOLME as well as silymarin into CCl_4-intoxicated rats resulted in significant improvement in all of these parameters compared with those of CCl_4-intoxicated rats. Administration of EtOAc and *n*-butanol fractions attenuated CAT activity but could not modulate the CCl_4-induced alterations in other antioxidant biomarkers (Table 3).

Table 3

Effect of different fractions of *M. oleifera* leaves methanol extract and silymarin on oxidant/antioxidant markers in liver homogenate of rats with CCl_4-induced hepatotoxicity (Means±SE, *n*=5).

| Groups	GSH (mM/g tissue)	GST (U/g tissue)	Catalase (U/g tissue)	Lipid peroxide by-products (nM/g tissue)
Control	7.04 ± 0.13[a]	116.99 ± 1.03[a]	67.82 ± 0.93[a]	56.39 ± 0.70[f]
CCl_4 alone	3.57 ± 0.14[d]	85.13 ± 0.93[e]	27.90 ± 1.05[e]	372.18 ± 9.54[b]
n-hexane	5.46 ± 0.07[b]	111.17 ± 2.79[bc]	64.03 ± 1.57[a]	137.25 ± 0.47[e]
DCM	3.90 ± 0.20[d]	106.28 ± 1.24[d]	57.81 ± 1.01[b]	154.59 ± 3.25[c]
EtOAc	2.84 ± 0.23[e]	74.62 ± 1.50[f]	35.83 ± 0.84[d]	393.89 ± 1.56[a]
n-butanol	2.62 ± 0.11[e]	77.02 ± 0.49[f]	44.23 ± 1.16[c]	386.00 ± 3.83[a]
Aqueous	4.93 ± 0.24[c]	110.31 ± 0.62[c]	32.40 ± 0.40[d]	152.56 ± 1.64[cd]
Silymarin	5.09 ± 0.17[bc]	115.09 ± 1.22[ab]	57.20 ± 3.42[b]	142.57 ± 1.10[de]

Means with different superscripts in the same column are significantly different at $P<0.05$. GSH: Glutathione reduced. GST: Glutathione-s-transferase. DCM: Dichloromethane. EtOAc: ethyl acetate.

3.2.4. Histopathological findings

Microscopic examination of livers of normal rats showed normal hepatic architecture with normal central vein, portal tract and radiating hepatic cords (Figure 2A). Livers of CCl_4-intoxication showed massive and diffuse fatty change, cellular necrosis, collapsed blood sinusoids, infiltration of mononuclear inflammatory cells, perilobular, and pericentral vein fibrous connective tissue (C.T.) accompanied with presence clusters of hemosiderin laden macrophages. In portal area marked fibrous C.T. proliferation associated with focal aggregation of mononuclear cells was recorded. There were dilatation and congestion of hepatic blood vessels (Figure 2B). Liver of CCl_4–intoxicated rats treated with *n*-hexane or DCM fraction of MOLME showed almost normal hepatic architecture, moderate degree of fatty change, activation of Kupffer cells with mild inflammatory cell infiltration. There is no fibrous C.T. proliferation around the central vein and massive accumulation of mononuclear inflammatory cells in portal area (absence of fibrosis) and hepatic sinusoids. The cellular swelling without fatty change and rare centrilobular necrosis were reported. There was activation of Kupffer cells (Figure 2C and 2D). Treatment of CCl_4–intoxicated rats with EtOAc fraction of MOLME showed severe and diffused scattered fatty change (steatosis) accompanied with individual cell necrosis. Multiple areas of hepatic cell necrosis associated with dissociation of hepatic cords, collapsed hepatic sinusoids and inflammatory cell aggregations were observed. Also, there were dilatation and congestion of hepatic blood vessels. In the portal tract, marked fibrous C.T. proliferation accompanied by aggregation of

inflammatory mononuclear cells was seen (Figure 2E). Treatment of CCl$_4$–intoxicated rats with n-butanol fraction showed extensive centrilobular fatty change associated with a proliferation of Kupffer cells, haemosidrosis, individual cellular necrosis, and engorged hepatic blood vessels. The portal tract showed fibrous C.T. proliferation (fibrosis) accompanied by aggregation of inflammatory mononuclear cells (Figure 2F). Treatment of CCl$_4$–intoxicated rats with aqueous fraction showed mild to moderate centrilobular fatty change of hepatic cell and infiltration of inflammatory mononuclear cells mainly lymphocytes and macrophages (Figure 2G). Treatment of CCl$_4$–intoxicated rats with silymarin, normal hepatic architecture was seen in the most of examined cases. The hepatocytes were relatively swollen with granular cytoplasm, vesicular nuclei and prominent nucleoli. Activation of Kupffer cells was also seen. In few cases mild to moderate degree of fatty change of centrilobular hepatic cells associated with few numbers of mononuclear inflammatory cells in hepatic parenchyma were observed (Figure 2H).
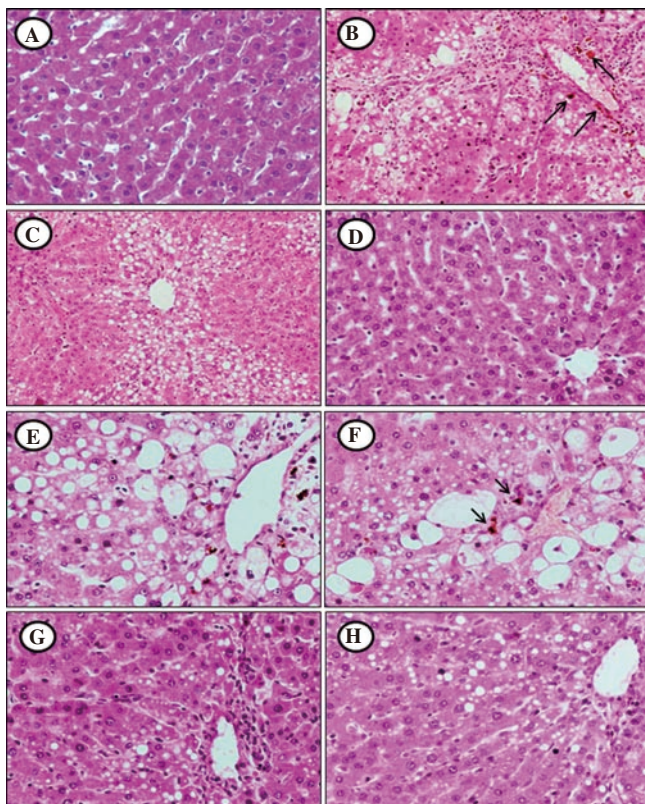
Figure 2. Liver sections of (A) normal rat, CCl$_4$–intoxicated rat treated with (B) vehicle, (C) n-hexane fraction, (D) dichloromethane fraction, (E) ethyl acetate fraction, (F) n-butanol fraction, (G) aqueous fraction and (H) silymarin. (H&E, ×200).

4. Discussion

In the present study, the MOLME was fractionated and its components were identified by comparing their mass spectra and retention time with those of the NIST and WILEY library. The component of the methanol extract of Moringa plant has been reported to contain 100 compounds[20]. However, in this work, the constituents of different fractions of the hydromethanol extract were analyzed for the first time. The n-hexane, DCM, EtOAc, n-butanol and the aqueous fraction contained 17, 22, 23, 19 and 32 compounds of which 7, 12, 8, 15, and 18 compounds were of more than 1% each, respectively. The chemical structure of each compound was identified. This information is valuable for isolation, purification and further evaluation of their therapeutic efficacy and safety for human and animals. In continuation of this information, the antioxidant and hepatoprotective effects of these fractions were tested.

Although the *in vitro* antioxidant effect of crude methanol extract of *M. oleifera* leaves has been previously reported[17], the present results markedly revealed, for the first time, that n-butanol (89.9%) and EtOAc (88.9%) fractions exhibited the strongest *in vitro* free radicle scavenging activity against DPPH. Similar antioxidant effect of *M. oleifera* was previously reported[21]. The CCl$_4$ toxicity was characterized by a significant increase in ALT and AST activity and total cholesterol and LPx levels. Moreover, CCl$_4$ decreased the activities of both hepatic GST and CAT and the concentration of total proteins and GSH. The mechanism of CCl$_4$ intoxication may be attributed to a direct toxic effect of CCl$_4$ metabolites or to a peroxidative degeneration of membrane lipids causing the development of hepatic steatosis, increased serum ALT and AST activities and elevated triglycerides level[22]. The CCl$_4$-oxidative injury caused disruption of the hepatocellular plasma membrane, inhibition of protein synthesis and release of hepatic transaminases[23]. The activity of ALT and AST has long been used as a good marker of assessment liver function. These enzymes are released from damaged hepatocytes and their activity in plasma is increased. Their activity is therefore used as a useful marker to the extent and type of hepatocellular damage[24]. Moreover, CCl$_4$-induced fatty degeneration is responsible for the reported increased serum triglycerides level[25] and the decreased albumin level[23].

The antihepatotoxic effects of different fractions of MOLME were marked and comparable to that of silymarin. This is based on the significant alleviation of the CCl$_4$-induced alteration of liver enzymes, triglycerides and glucose. Previous studies have demonstrated that crude MOLME exhibited hepatoprotective effect against different hepatotoxic chemicals such as antitubercular drugs[26], diclofenac[27], acetaminophen[28] and CCl$_4$[16]. In this study, additional evidence revealed that n-hexane, DCM and aqueous fractions are more powerful in decreasing the CCl$_4$-elevated AST, ALT and total cholesterol. These three fractions were also the more powerful in increasing the level of GSH and LPx and the activity of GST and CAT which have been reduced in CCl$_4$-intoxicated rats. It was observed that the decrease in the activity of ALT was more pronounced than the decrease in AST indicating that the effect of different fractions of MOLME is more specific on the hepatocytes which are the most exclusive source of ALT than AST. The decreased activity of liver enzymes in serum of treated rats may be attributed to a membrane stabilizing effect. This

effect could be due to its content of total phenolics or flavonoids which reduce lipid peroxidation in rat liver[29] and/or steroids and triterpenoids[30] which have an antioxidant activity. The methanol extract of *M. oleifera* leaves was reported to contain flavonoids, saponins and unsaturated sterols and/or triterpenes[16]. Phenolic compounds were also separated from *M. oleifera*[29,31] which were reported to be responsible at least for its antioxidant activity. Many compounds exert their protective action by decreasing the production of CCl$_4$ derived-free radicals or by their own antioxidant effect[32]. The *n*-butanol and EtOAc fractions of MOLME appeared to have the strongest *in vitro* free radicle scavenging activity but exhibit less *in vivo* effectiveness against CCl$_4$-induced liver injury in rats. In this respect, the free radical scavenging activity of plant extracts is usually dependent on the solvent used for extraction[33]. This fact could explain the difference in antioxidant potency among different fractions. Moreover, the antioxidant phytochemicals such as phenolics are subjected to numerous biochemical reactions along ingestion, digestion, and absorption by the organisms. Moreover, the co-ingestion of other nutrients can affect their bioavailability in relation to the ingested dose[30]. Therefore, the *in vitro* antioxidant effects are not usually consistent with the *in vivo* properties[34]. *M. oleifera* has been reported to lower the serum total lipids, an effect which could account for the triglycerides and cholesterol lowering effect[6]. On the other hand, the anti-hepatotoxic effect may be attributed to a number of active constituents reported from the phytochemical analysis such as *L*-valine, which has been suggested to have therapeutic potential in liver cirrhosis[35]. Moreover, ethyl caffeate reported in the phytochemical analysis was suggested to reduce the degree of liver injury when administered intraperitoneally in rats[36]. In correlation to the biochemical and antioxidant biomarkers, liver of CCl$_4$-intoxicated rats treated with the *n*-hexane, DCM and aqueous fraction of methanol extract of *M. oleifera* showed mild to moderate degree of fatty change, mild inflammatory cell infiltration, less severe hepatic lesions and mild to moderate vacuolar degeneration of hepatic cell parenchyma and C.T. proliferation. In contrast to rats treated with EtOAc and *n*-butanol fraction which showed marked and diffuse fatty change (steatosis), cellular necrosis and inflammatory cell aggregations with fibrous C.T. proliferation, extensive centrilobular fatty change associated with proliferation of Kupffer cells, hemosidrosis, cellular necrosis and inflammatory cells infiltration. In this concern, improvement of liver histopathological picture by MOLME has been previously reported on liver damage induced by CCl$_4$[16] and other hepatotoxic drugs[26] in rats. In this study the *n*-hexane, DCM and aqueous fraction of methanol extract of *M. oleifera* are the best to ameliorate CCl$_4$-induced pathological changes.

Conflict of interest statement

The authors declare no conflict of interests.

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